STATUS OF CLAIMS

1-13 (canceled)

O.K. to enter Arun Ms. Chakrabarti 6/20/03

14. (previously amended) A method for validating the effect of a candidate gene that is expressed in a mammalian neural cell of interest, said method comprising:

(a) producing a candidate dsRNA which comprises at least 100 nucleotides of said candidate gene;

(b) introducing said candidate dsRNA into a reference mammalian neural cell; and

(c) validating the effect of said candidate gene by detecting an alteration in a cellular activity or a cellular state in said reference mammalian neural cell, wherein said alteration is the result of specific attenuation of mRNA corresponding to said candidate in said reference mammalian neural cell, indicating that said candidate gene plays a functional role in mammalian neural cells.

15. (previously amended) The method of claim 14, wherein said step of producing the candidate dsRNA comprises:

producing a cDNA corresponding to said candidate gene from an mRNA of said mammalian neural cell of interest; and producing the candidate dsRNA from said cDNA.

16. (canceled)

17. (previously amended) The method according to Claim 15, further comprising:

producing a plurality of candidate cDNAs from said mammalian neural cell of interest;

producing a plurality of candidate dsRNA which comprise at least 100 nucleotides of said candidate cDNAs;

introducing each of the candidate dsRNA into a plurality of separate reference mammalian neural cells having a gene expression similar to said mammalian neural cell of interest;

and validating the effect of said candidate genes by testing for alterations in a cellular activity or a cellular state in said reference mammalian neural cell that result of attenuation of mRNA corresponding to said candidate in said reference mammalian neural cell, wherein detection of said alterations is indicative that said candidate gene plays a functional role in said mammalian neural cells of interest.

18. (canceled)

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19. (previously amended) The method of claim 17, wherein said step of producing a plurality of candidate cDNAs comprises:

producing double-stranded cDNA from mRNA by reverse transcription;

producing cDNAs of a similar length by digesting said cDNA with a restriction enzyme; and

producing a plasmid or PCR fragment from said cDNA after said digesting step.

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20. (previously amended) The method of claim 19, wherein the candidate dsRNA is produced by transcribing said plasmid cDNA or PCR fragment.

21. (canceled)

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22. (original) The method of claim 19, wherein the restriction enzyme is selected from the group consisting of Dpn1 and Rsa1.

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23. (previously amended) The method of claim 17, wherein said step of producing the plurality of candidate dsRNAs comprises: selecting a candidate cDNA that is expressed at a detectably different level with respect to said reference mammalian neural cell and said mammalian neural cell of interest, and said reference mammalian neural cell and said mammalian neural cell of interest differ with respect to a cellular characteristic that is detectable by said step of testing for alterations in a cellular activity or a cellular state.

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24. (previously amended) The method of claim 23, wherein the candidate cDNA is selected from a normalized library prepared from said reference mammalian neural cells or said mammalian neural cell of interest and is present in low abundance in the normalized library.

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25. (previously amended) The method of claim 23, wherein the candidate cDNA is a differentially expressed cDNA selected from a subtracted library that is enriched for cDNAs that are differentially expressed with respect to said reference mammalian neural cells or said mammalian neural cell of interest.

26. (canceled)

10 27. (previously amended) The method of claim 23, wherein said step of selecting the candidate cDNA comprises:

preparing a tester-normalized cDNA library from test cells; a driver-normalized cDNA library from control cells; a tester-subtracted cDNA library which is enriched in one or more genes that are up-regulated with respect to the test cell and the control cell, and a driver-subtracted cDNA library which is enriched in one or more genes that are down-regulated with respect to the test cell and the control cell; and

selecting a cDNA from the normalized libraries by contacting cDNAs from the tester-normalized cDNA library with labeled probes derived from mRNA from test cells and contacting cDNAs from the driver-normalized cDNA library with labeled probes derived from mRNA from control cells under conditions whereby probes specifically hybridize with complementary cDNAs to form a first set of hybridization complexes; and detecting at least one hybridization complex from the first set of hybridization complexes to identify a cDNA that is present in low abundance.

28. (canceled)

11 29. (previously amended) The method of claim 23, wherein said step of selecting the candidate cDNA comprises:

preparing a tester-normalized cDNA library from test cells; a driver-normalized cDNA library from control cells; a tester-subtracted cDNA library which is enriched in one or more genes that are up-regulated with respect to the test cell and the control cell, and a driver-subtracted cDNA library which is enriched in one or more genes that are down-regulated with respect to the test cell and the control cell; and

selecting a cDNA from the subtracted libraries by contacting cDNAs from the tester-subtracted cDNA library and contacting cDNAs from the driver-subtracted cDNA library with a population of labeled probes under conditions whereby probes from the population of probes specifically hybridize with complementary cDNAs to form a second set of hybridization complexes, and wherein the population of labeled probes is derived from mRNA from test cells and control cells; and detecting at least one hybridization complex from the second set of hybridization complexes to identify a cDNA that is differentially expressed above a threshold level with respect to the subtracted libraries.

12 30. (previously amended) The method of claim 23, wherein the cellular characteristic is cell health, the test cell is a diseased neural cell and the control cell is a healthy neural cell, and the candidate gene is suspected of correlation with a disease.

13 31. (original) The method of claim 30, wherein the test cell is obtained from a mammal that has had a stroke or is at risk for stroke.

32-33. (canceled)

14 34. (previously amended) The method of claim 23, wherein the cellular characteristic is cellular differentiation and the candidate gene is suspected of correlation with control of cellular differentiation.

15 35. (previously amended) The method of claim 23, wherein the candidate gene is endogenous to said mammalian neural reference cell.

16 36. (previously amended) The method of claim 23, wherein the candidate gene is an extrachromosomal gene in said mammalian neural reference cell.

37-42 (canceled)

17 43. (previously amended) The method of claim 30, wherein said mammalian neural reference cell is a neuroblastoma cell.

44. (canceled)

18 45. (previously amended) The method of claim 44, wherein said mammalian neural reference cell has increased sensitivity to N-methyl-D-aspartate, β-amyloid, peroxide, oxygen-glucose deprivation, or combinations thereof, relative to a normal mammalian neural cell.

19 46. (previously amended) The method of claim 45, wherein the detecting step comprises detecting a decrease in cellular sensitivity to N-methyl-D-aspartate, β-amyloid, peroxide, oxygen-glucose deprivation, or combinations thereof, relative to a normal mammalian neural cell.

20 47. (original) The method of claim 17, wherein the detecting step comprises detecting modulation of ligand binding to a protein.

48-50 (canceled)

21 51. (previously amended) The method of claim 14, wherein the determining step comprises determining whether the protein encoded by the candidate gene binds to another protein to form a coimmunoprecipitating complex.

22 52. (previously added) The method of claim 14, wherein the candidate dsRNA is at least 500 nucleotides in length.

23 53. (previously added) The method of claim 14, wherein the candidate dsRNA is between 500 and 1100 nucleotides in length.

24 54. (previously added) The method of claim 14, wherein said mammalian neural cell of interest is a glial cell.

25 55. (previously added) The method of claim 14, wherein said reference mammalian neural cell is a glial cell.

APPLICANTS' UNEXPECTED RESULTS

Applicants respectfully submit that the present claims are not made obvious by any the cited combination of art. The present claims are drawn to the use of dsRNA comprising at least 100 nucleotides of a candidate gene, for the specific attenuation of expression of genes in mammalian neural cells.

Prior to Applicants' invention, it was known in the art that the introduction of dsRNA of longer than 30 bp into mammalian cells induced an undesirable interferon response. As discussed by Der *et al.* (1995) (attached), double stranded RNA induces interferon expression, which leads to general suppression of gene expression in cell death. These undesirable side effects meant that long double stranded RNA could not be used to study gene attenuation in mammalian cells. The only dsRNA useful in mammalian cells is short dsRNA, usually about 20-25 bp in length.

It would have been expected that neuronal cells would also show an interferon response with the introduction of long dsRNA, because the prior art publication by Ward *et al.* (1995) J. Neuroimmunology 58:145-155 (attached), stated "in primary neuronal cultures following exposure of these cells to known IFN-inducing agents, including double-stranded RNA . . . it was found that neurons rapidly express high levels of IFN-β."

Applicants have attached herewith a research article published by co-inventors Li Gan, Kristen Anton and Mirella Gonzalez-Zulueta (Gan *et al.* (2002) J. Neuroscience Methods 121:151-157), which discusses the unexpected findings of the present invention.

It would have been expected by one of skill in the art that the use of dsRNA of at least 100 nt in length would lead to interferon expression in neuronal cells. And yet, as shown by Applicants, (specification, page 56, last paragraph,ff) specific attenuation was observed in neuronal cells for GFP (green fluorescent protein), and for PARP, both in the levels of the protein produced, and in the functional effects mediated by the protein. Cells transfected with dsPARP-N showed significant protection against OGD-induced cell death compared with mock-transfected cells and those transfected with dsGFP. These results validate the use of RNAi in the analysis of the role of novel and known genes in neurons.

DEFICIENCIES OF THE PRIOR ART

The prior art does not teach or suggest the presently claimed invention. Leptin, U.S. 6,135,942, has been cited against the present claims. The teachings of Leptin are specific to Drosophila (insect) cells. Insect cells do not make interferon. Therefore, the adverse effects of introducing long dsRNA into mammalian cells is not observed with insect cells. Because Drosophila

cells do not share the biology of mammalian cells with respect to induction of interferon by dsRNA, Leptin does not teach or suggest the function of RNAi in mammalian cells.

Der *et al.* has been cited in combination with Leptin. Der *et al.* do not teach the use of double stranded RNA, and therefore cannot teach the presently claimed methods. Der *et al.* teach the use of anti-sense RNA, which is a single stranded RNA, not double stranded. In mammalian cells, the introduction of single stranded RNA does NOT induce an interferon response. Therefore, the effects of double stranded and single stranded RNA are different in mammalian cells.

One of skill in the art is not informed of the present invention by the teachings of Der combined with Leptin.

Petryshyn, U.S. 6,124,091, has been cited in combination with Leptin. Applicants respectfully submit that Petryshyn does not suggest the present invention, alone or in combination with Leptin. Petryshyn teaches an RNA molecule that activates PKR, which is part of the interferon response observed in mammalian cells. Double stranded RNA induces interferon expression, and expression of the double-stranded-RNA dependent kinase PKR mediates interferon activity.

Example 1 of Petryshyn discloses the cDNA synthesis of R-RNA, and does not disclose RNA interference.

Example 11 of Petryshyn describes the use of short anti-sense oligonucleotides, which are single stranded. The RNA used in the present methods are double stranded RNA. As explained above, double stranded and single stranded RNA differ in their ability to induce an interferon response. Because the effects of single stranded RNA are distinct from that of double stranded RNA, one does not predict the activity of the other.

Applicants respectfully submit that Petryshyn does not make obvious the presently claimed invention, and does not remedy the deficiencies of the primary reference, Leptin.

The citation of Kreitman *et al.* is provided for teaching the use of the restriction enzyme RsaI. Applicants respectfully submit that the use of the restriction enzyme is not relied upon for patentability, but is cited as one embodiment of the invention. Kreitman *et al.* does not remedy the deficiencies of the primary reference, which fails to teach or suggest the use of dsRNA of greater than 100 nt in neuronal cells to specifically attenuate gene expression.

Villeponteau *et al.* fail to remedy the deficiencies of the primary reference by failing to teach the use of dsRNA of greater than 100 nt in neuronal cells to specifically attenuate gene expression. The reference teaches the generation of libraries, but not the use of dsRNA in gene attenuation.

Staddon *et al.* does not teach the use of long dsRNA to attenuate gene expression in neuronal cells.

In view of the above amendments and remarks, Applicants respectfully submit that the present invention meets the requirements of 35 U.S.C. 103. Withdrawal of the rejections is requested.

Applicants submit that all of the claims are now in condition for allowance, which action is requested. If the Examiner finds that a Telephone Conference would expedite the prosecution of this application, he is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any other fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number AGYT-013CIP.

Respectfully submitted,

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